

3D perfusion bioreactor technologies for hepatic stem cell maintenance, differentiation and expansion

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Hybrid extracorporeal liver support with bioreactors is an option to assist liver transplantation therapy. Bioreactors are also in development for the expansion of hepatic progenitor cells and give a perspective for the development of innovative cell transplantation methods.

Bioartificial liver (BAL) bioreactor devices have undergone a rapid evolution in the last decade. "First generation" BAL devices utilize commercial, off-the-shelf filtration and plasma-pheresis units for the bioreactor core. Some clinical work with clinical BAL systems was performed using such commercial available hollow fiber cartridges for dialysis or plasma-separation (two compartment systems). "Second-generation" bioreactors were developed as specialized units intended to address the shortcomings of commercial available cartridges.

Bioreactors that are currently used for primary liver cell culture and clinical liver support are hollow fiber based and exhibit two functional compartments. Xu et al. pointed out, that four bioreactor compartments are necessary to enable integral oxygenation and distributed mass exchange with low gradients.

Sophisticated bioreactors enable a spontaneous re-assembly of primary cells inoculated into a bioreactor and their establishment of a scaffold or biomatrix. A homogeneous mix of adult liver cells from organ collagenase digestion containing parenchymal hepatocytes, non-parenchymal cells (such as sinusoidal endothelial cells, stellate cells, and liver progenitor cells) can restructure after injection into specific bioreactors to form well-defined liver structures, such as neo-sinusoidal structures and neo-spaces of Dissé, reminiscent of the native liver. Moreover, adult liver progenitors restructure anatomical structures resembling the Canal of Hering, the putative liver stem cell niche.

Stem cells or adult liver progenitor cells may be the ideal cell source for the development of cell-based liver therapies since they may have therapeutic benefit in the support, repair, and regeneration of damaged tissues. To realize the full therapeutic potential of stem cells, many investigators are seeking *in vitro* methods for maintaining the unspecialized state of stem cells in bioreactors, promoting the proliferation of stem cells, and directing the differentiation of stem cells to specialized hepatic cells. The development of bioreactor technologies to address these needs would also result in system of great interest for expansion of embryonal stem cells and their possible differentiation towards hepatic cells.

The current challenge that prevents stem cells from use in these applications is directing the full differentiation of their progeny *in vitro*. In order to further develop new therapies, adult and embryonic stem cell research focuses on maintenance, proliferation and differentiation, and tissue formation *in vitro*. Numerous groups already work on derivation and characterization of specific stem cell lineages, but the underlying mechanisms are only partly understood.

Several fundamental questions remain to be answered, e.g.:

- Can we control proliferation/differentiation of selected stem cells *in vitro*?
- Can we maintain the genotype/phenotype stability of selected stem cells *in vitro*?
- Can specific micro-environmental conditions be used to control *in vitro* maintenance of selected stem cells?
- Can, after inoculation of selected isolated stem cells, tissue restructuring be achieved by the cells themselves?
- Can tissue formation by selected stem cells be induced and controlled *in vitro*?
- Can we establish *in vitro* a tissue-density of a larger number of selected stem cells without central necrosis?
- Can a reproducible proliferation/differentiation and utilization of selected stem cells be achieved *in vitro*?
- Can specific macro-environmental conditions, such as 3D high-density co-culture with integrated oxygenation and decentralized mass exchange, be used to control *in vitro* maintenance of human embryonic stem cells in a larger cell mass?
- Can a phenotypic stabilization of selected stem cells and the maintenance of a liver progenitor cell pool be achieved by 3D high-density co-culture with integrated oxygenation and decentralized mass exchange?
- Does the integration of a more physiological tissue macro-environment, e.g. by hollow fiber membranes into a growing cell mass result in a genotypic/phenotypic stabilization of proliferating stem cells?
- Can we maintain the stability of human embryonic stem cells *in vitro*, while they are proliferating as a larger mass and differentiating towards hepatic cells?

For several topics, experimental animal source or biopsied human tissue, as well as conventional Petri-dish *in vitro* culture methods seem to provide appropriate tools for investigation. However, investigations focusing on the impact of exogenous factors could benefit from the use of purpose-built bioreactors that enable 3D high-density perfusion co-culture. There is a considerable need for such *in vitro* stem cell systems, since the stem cell-derived tissues must be capable of stable and long-term integration; into a bioreactor or, after transplantation, into existing physiological tissues, at least until they are replaced by the body's own tissue repair process, or permanently if self-repair is not possible. At least some of such studies require reproducible and controllable *in vitro* conditions.